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cells, into a population which represents the smallest 30% of the cells in the sample and into a population which comprises larger cells, wherein the population with the smallest 30% of the cells comprises epidermal stem cells.

Remarks

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 39-40 are added. Claims 1-40 are pending.

New claims 39-40 are supported by originally-filed claims 1-2 and at page 3, lines 19-25 and Example 1 in the specification.

The 35 U.S.C. § 112, Second Paragraph, Rejection

The Examiner rejected claims 1-27 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. This rejection is respectfully traversed.

Although the Examiner asserts that the claims do not set forth essential steps or experimental parameters, the Examiner has failed to particularly indicate which steps or parameters are missing. Applicant's Representatives await clarification on this issue, however, it is Applicant's position that one of ordinary skill in the art would understand the metes and bounds of claims directed to a method to prepare isolated epidermal stem cells which employs a population comprising epidermal stem cells and a separation step to yield at least two populations. For example, a separation step can include separating a population of mammalian epidermal cells into a population comprising epidermal stem cells and into a population of cells that does not comprise epidermal stem cells (claims 1, 16 and 39), or into a population which represents the smallest 30% of the cells in the sample, i.e., cells which include epidermal stem cells, and into a population which represents larger cells (claims 2 and 40). In one embodiment, prior to separation, the population of mammalian epidermal cells is contacted with a first agent under conditions effective for viable cells to retain the first agent and with a second agent under conditions effective for non-viable cells to retain the second agent (claim 16; see Example 1).

An exemplary first agent is a Hoechst dye and exemplary second agents include propidium iodine and pyronidine iodine (see claims 7, 9, 22, 24, and 39-40).

Thus, the metes and bounds of the claims would be clear to the art worker in the absence of Applicant's specification or, alternatively, in view of Applicant's specification. Hence, withdrawal of the § 112(2) rejection is respectfully requested.

The 35 U.S.C. § 102(b) Rejections

The Examiner rejected claims 15 and 27 under 35 U.S.C. § 102(b) as being anticipated by Scadden (U.S. Patent No. 5,827,742) or Eriksson et al. (U.S. Patent No. 5,423,778). These rejections are respectfully traversed.

The Examiner acknowledges that the claimed methods are novel. Nonetheless, the Examiner asserts that Scadden and Eriksson et al. teach isolated epidermal stem cells. However, Scadden discloses the isolation of quiescent pluripotent human hematopoietic progenitor cells which are substantially free of mature human lymphoid and myeloid cells (column 2, lines 13-17). It is also disclosed that the quiescent pluripotent human hematopoietic progenitor cells are isolated from a mammalian hematopoietic cell suspension such as from bone marrow, peripheral blood, umbilical cord blood or fetal liver cells by contacting the mononuclear cell suspension with an antibody specific for a cell surface antigen expressed by immature hematopoietic precursor cells, e.g., c-kit, CD34 and Thy-1 (column 2, lines 30-38). Note that those surface antigens are not known to be present on epidermal stem cells. It is further disclosed that the mononuclear cells or antibody-treated mononuclear cells are cultured with at least one anti-metabolite and one early acting growth factor (column 2, lines 44-47) and that the resulting cells can be characterized by staining with propidium iodide and then with Hoechst 3342 prior to FACS analysis (column 11, lines 1-20). Scadden does not mention epidermal stem cells.

Eriksson et al. describe a method whereby a wound chamber system is employed to deliver a viral vector or plasmid containing a transgene to keratinocytes, e.g., to cells including those having a high percentage of epidermal stem cells (abstract). The wound chamber system is disclosed as providing direct *in vivo* gene transfer to exposed cells in an open wound (column 2, lines 9-11). Eriksson et al. note that skin stem cells, which are located in the hair follicles and

basal layer of the skin, are used to enhance long term survival of the transgenic cells (column 2, lines 19-21).

To prepare keratinocytes, Eriksson et al. disclose that dermis is treated with dispase and then with trypsin, after which fibroblasts are removed (column 3, lines 33-35). Such a method results in a population of basal cells including epidermal stem cells, which population is the proliferative population in the epidermis (see Lindberg et al., Differentiation, 45, 230 (1991), a copy is enclosed herewith). Example 1 discloses that keratinocytes were harvested from two Yorkshire pigs by partial thickness skin excision, dispase treatment, trypsin treatment, and mincing to yield a single cell suspension (column 8, lines 4-8). To prepare transgenic cells, it is disclosed that the resulting cells were grown to subconfluence and then infected with a retroviral vector (column 8, lines 9-11). The transduced cells were transplanted to open wounds and vector gene expression monitored (column 8, lines 14-33). Based on the transient expression of a marker gene present in the vector, it was concluded that dispase separation of dermis from epidermis did not allow for harvesting of a sufficient amount of epidermal stem cells (column 8, lines 32-35). It is also disclosed that results were improved by using a higher percentage of epidermal stem cells obtained from hair follicles (column 8, lines 41-43).

Eriksson et al. provide no evidence that the cells obtained after dispase/trypsin treatment and fibroblast removal include epidermal stem cells, much less what percent of those cells are epidermal stem cells. And although Eriksson et al. note that hair follicles contain a higher percentage of epidermal stem cells, Eriksson et al. fail to describe a method which results in a substantially pure population of epidermal stem cells.

Hence, neither Scadden nor Eriksson et al. anticipate claim 15 or 27.

Therefore, withdrawal of the § 102(b) rejections is respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

JACKIE R. BICKENBACH ET AL.,

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6959

Date August 7, 2002 By _____
Janet E. Embretson
Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 7th day of August, 2002.

Name Candis B. Buending

Signature _____